修 士 学 位 論 文

先駆樹種オオバアサガラの生残や成長に環境要因がおよぼす影響：野外調査と栽培実験による検討(英文)

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論文題名：先駆樹種オオバアサガラの生残や成長に環境要因がおよぼす影響：
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英文題名：Field and experimental studies of the effects of environmental factors on survival and growth of a pioneer tree, *Pterostyrax hispida*

Regeneration of trees is affected by repeated disturbances such as flood or debris flow especially in a mountain valley and, therefore, a mountain valley often develops specific vegetation. A pioneer tree, *Pterostyrax hispida* is common in this type of vegetation and recently increases its number. The increases are often attributed to its unpalatability of sika deer. Because sika deer avoid *P. hispida* plants, their seedlings and saplings would facilitate recruitment of other palatable plants that have been severely grazed by sika deer. In this thesis, I investigated the effects of microenvironments on the survival and growth of *P. hispida* to detect safe sites of seedlings of *P. hispida*.

First, I investigated the effects of microenvironments on distribution of seedlings/saplings of *P. hispida* in a mountain valley in the University of Tokyo Chichibu Forest (Chapter 2). Six line transects were established perpendicularly to the River Oochi from the river flow to mountain ridge and 76 quadrats (2 m × 2 m) were randomly distributed along the transects in 2017. Numbers of *P. hispida* plants, topography and canopy openness of the quadrats were measured in 2017 and/or 2018. A plastic net bag including 300 seeds of *P. hispida* with 2 L vermiculite were put beside the 39 quadrats in November 2017 and the germinated seedlings in each bag were counted once in a month from April to October in 2018. Topography and canopy openness gradually changed from the flow to the mountain ridge. Fourteen

seedlings were observed in the most germinated bag. Occurrence of *P. hispida* seedlings did not converged into a certain environmental condition in the quadrats. Very limited number of seedlings and low germination percentage suggested the necessity of a large amounts of seeds for seedlings establishment.

Second, I investigated the effects of temporally heterogeneous flood on the survival and growth of current-year seedlings in a growth experiment (Chapter 3). A current-year seedlings of *P. hispida* was grown in a pot under the flood treatment that included four levels; submerged a pot for 2 days and emerged the pot for 2 days in the 2 days flood treatment, submerged a pot for 4 days and emerged the pot for 4 days in the 4 days flood treatment, submerged a pot for 8 days and emerged the pot for 8 days in the 8 days flood treatment and the control treatment in which a pot was unsubmerged and watered every 2 days. The flood treatment did not affect plant mortality and leaf dynamics although the 8 days flood treatment was harsh condition. Indeed, the risen water that stood 50 cm above normal level did not last above eight days between 2012 and 2016 except for only once in the River Oobora, near the University of Tokyo Chichibu Forest. Only plant growth was significantly influenced by the flood treatment. Especially, the flood period in this experiment reduced the increment of seedling biomass but not seedling survival of *P. hispida*.

Third, I investigated the effects of soil surface area and soil particle size on the survival and growth of current-year seedlings of *P. hispida* and *Euptelea polyandra* (Chapter 4). A current-year seedlings of *P. hispida* or *E. polyandra* was grown in a pot with same pot-volume under different combination of pot diameter (narrow, middle or wide) and soil particle size (small or large). In both species, plant mortality in a pot with narrow diameter was significantly higher than those with wider diameters, but plant growth was significantly larger in a pot with wide diameter than that with narrow diameter. These results and harvested root

morphology suggest that *P. hispida* and *E. polyandra* would extend their roots horizontally. Actually, horizontal root growth of both species was observed at the field site. Both species would prefer thin and wide-opened soil surface rather than deep and narrow-opened soil surface under enough water supply.

In conclusion, seeds of *P. hidpida* would not require specific environments to germinate but amounts of dispersed seeds are suggested to be important for *P. hispida* to establish because of low percentage of germination. If geminated seedlings are submerged for ordinally flooding period, they would survive. If the germinated seedlings establish even on thin soil layers, they would spread their root systems and survive.

Contents

Chapter 1

General Introduction

Regeneration of trees is affected by repeated disturbances such as floods or debris flow especially in a mountain stream. Disturbances create various topographies and microenvironments (Sakio, 1997; Suzuki et al., 2002), therefore, the microenvironments of mountainous streams are more heterogeneous especially in soil and light conditions than surrounding forests above the valley bottoms. Environmental heterogeneity at various scales, thus influences establishment of trees. Ground surface conditions affect seedlings. Fates of the seedlings dispersed on sands would differ from those on gravel. In addition to the soil medium, shapes of soil such as soil depths or surface areas, also influence fates of seedlings. Furthermore, if seeds are dispersed beside a stream flow, seedlings would be submerged by flooding (Sakio, 2005; Higa, Moriyama, & Ishikawa, 2012). Habitat preference of seedling establishment is highly species specific, hence a mountain valley often develops specific vegetation (Suzuki et al., 2002).

A pioneer tree, *Pterostyrax hispida*, is one of the common species in a mountain valley (Oohashi et al., 2017) and is often distributed in disturbed areas such as forest-road verges and riverbanks. Number of *P. hispida* is increasing in mountainous areas in Kanto region (Ninomiya & Furubayashi, 2003) although most of plants disappeared due to sika-deer grazing except for unpalatable plant species (Takatsuki, 2009; Sakio et al., 2013). Because sika deer avoids *P. hispida* due to its unpalatability (Ninomiya & Furubayashi, 2003; Tamura, 2014; Tamura et al., 2016), seedings or saplings of *P. hispida* often facilitate recruitment of other palatable plants that have been severely grazed by sika deer (Ishida et al., 2008; Tamura, 2014;

Tamura et al., 2016). Therefore, environmental conditions of safe sites of *P. hispida* should be clarified for forest restoration in Kanto mountains.

This thesis reports the effects of environmental factors on survival and growth of a pioneer tree, *Pterostyrax hispida* to clarify its regeneration conditions. In Chapter 2, I investigated the effects of microenvironments on germination of *P. hispida* and the distribution of its seedlings and saplings in a mountain valley. In Chapter 3, I evaluated the effects of temporally heterogeneous floods on the survival and growth of current-year seedlings of *P. hispida*. In Chapter 4, I studied the effects of soil-particle sizes and pot diameters on the survival and growth of current-year seedlings of *P. hispida* and *Euptelea polyandra*. In Chapter 5, I concluded regeneration conditions of *P. hispida*.

Chapter 2

Effects of microenvironment on the distribution of seedlings/saplings of *Pterostyrax hispida* in mountain valley

Introduction

One of the central goals of ecology is to understand the mechanisms that determine distribution of plants. One of key factors to determine the distribution of plant species is seed limitation. Actually, differences in dispersal ability and chances of establishment are important mechanisms to shape a plant community. Niche differentiation by environmental filtering (Keddy, 1992) is another key factor to determine the distribution of plant species. Only a set of species can survive through a sieve of abiotic environments (Silvertown, 2004). Both seed limitation and environmental filtering play a critical role to determine the distribution of plant species (Fraaije et al., 2015).

In a mountain valley, fine-scale environments are very heterogeneous, which provide various niches for species to coexist. Microenvironmental heterogeneity is caused by frequent disturbances in a mountain valley. Floods after heavy rain and debris flows with different intensity repeatedly occur and make microtopography in a mountain valley complex. Diverse environments provide various niches for many types of plant species which develops specific vegetation in a mountain valley (Gregory et al., 1991; Suzuki et al., 2002). Therefore, a mountain valley with heterogeneous microenvironments offers more chances to coexist more species than a mountain ridge.

Recruitment of trees is sensitive to abiotic environments in a mountain valley and establishment of trees are especially affected by frequency and intensity of disturbances. Flood

frequency depends on distances and heights from river flow, which affects establishment of seedlings (Nakamura, Yajima, & Kikuchi, 1997; Sakio, 1997; Nakamura, Shin, & Inahara, 2007). Light conditions also determine seed germination and survivorship of seedlings (Seiwa & Kikuzawa, 1996; Kubo, Sakio, Shimano, & Ohno, 2004), and are very heterogeneous in a mountain valley because of various canopy conditions caused by fluvial processes. Therefore, the regeneration of trees is affected by heterogeneity of fine-scale environmental factors in a mountain valley.

In a mountain valley, previous studies on riparian forests mainly focused on late-successional tree species (Sakio, 1997; Kaneko, Takada, & Kawano, 1999; Kaneko & Kawano, 2002; Sakio et al., 2002; Kubo et al., 2004), while little is known about regeneration of pioneer trees. How do environmental factors affect regeneration of pioneer trees in a mountain valley? Especially, how do environmental factors affect seeds or seedlings of pioneer trees? Therefore, I investigated the effects of environmental factors on establishment processes of a pioneer trees in a mountain valley.

One of the pioneer tree species, *Pterostyrax hispida*, in a mountain valley is often observed in disturbed areas such as forest-road verges, and riversides (Oohashi et al., 2017). Number of *P. hispida* is increasing in mountainous districts in Kanto region (Ninomiya & Furubayashi, 2003) although many plant species are decreasing their numbers and biomass because of grazing by sika deer (Takatsuki, 2009; Sakio et al., 2013). The increase of *P. hispida* can be caused by the unpalatability of sika deer (Ninomiya & Furubayashi, 2003; Tamura, 2014; Tamura et al., 2016). Seedings or saplings of *P. hispida*, therefore, would facilitate recruitment of other palatable plants that have been severely grazed by sika deer (Ishida et al., 2008; Tamura, 2014; Tamura et al., 2016). However, we still do not know safe sites of seedling establishment of *P. hispida*. Thus, I studied the effects of microenvironments on regeneration of *P. hispida* in a

mountain valley.

In this study I investigated microenvironments and distribution of seedlings/saplings of *Pterostyrax hispida*. Study plots were established along the line transects that cross various microenvironments from the river to ridges. In this study, (1) I measured fine-scale topographies and light conditions of study plots. I predicted that microenvironments are heterogeneous. (2) I counted numbers of seedlings or saplings of *P. hispida* in study plots and evaluated the relationships between the number of seedlings/saplings and microenvironmental conditions. (3) I conducted seed-sowing experiment to test whether seeds of *P. hispida* were able to germinate under various microenvironments of the study plots. This experiment allowed me to test the hypothesis of environmental filtering at seed or seedling stages because I was able to reject the hypothesis of seed limitation. I predicted that distances from the river flow and light conditions would determine germination of *P. hispida*.

Methods

Study site

The study was conducted in the University of Tokyo Chichibu Forest (35°53′–58′ N,138°46′–139°0′ E) located in central Japan (540 - 640 m a.s.l.). The annual mean temperature is 9.6 ℃ and precipitation is 1722.5 mm in 2016 (Miscellaneous Information of The University of Tokyo Forests, 2018). Surroundings of the study site were secondary forests or plantation forests of *Cryptomeria japonica*.

Field methods

Six line transects (A-F) were established perpendicularly to the Oochi-river towards ridges in July 2017. Some mature trees of *P. hispida* occurred around the line transects. The length of

transect A was 50 m, B 24 m, C 25 m, D 37 m, E 23 m, and F 24 m, respectively. Seventy-six quadrats (2 m × 2 m) were randomly set on the transects; ten on transect A; five on transect B; five on transect C; eight on transect D; six on transect E; and five on transect F. Relative heights from the river flow were measured in all quadrats by a water level (water surface of continuous water always has the same height). To estimate a light condition in each quadrat, hemispherical photographs were taken at 1.3 m height at the center of each quadrat using a digital camera (CoolPix 7700; Nikon Corp., Tokyo, Japan) in September 2018. The hemispherical photographs were analyzed by the software (CanopOn2; http://takenaka-akio.org/etc/canopon2/index.html). Numbers of seedlings and saplings of *P. hispida* in each quadrat were recorded in August 2018.

Seed bag experiment

Seeds of *P. hispida* were collected in the University of Tokyo Chichibu Forest in October 2017 and immature seeds were removed from the collected seeds. A seed bag (23 cm × 46 cm) made of plastic net to include three hundred seeds of *P. hispida* and 2 L vermiculite, was placed beside the 39 of 76 quadrats in November 2017. Seedlings germinated in a seed bag were counted from April to October once in a month in 2018. All counted seedlings were removed from the seed bag immediately after the census to determine when seedlings germinated.

The 33 seed bags were brought back to the laboratory after the census in October 2018, and 6 seed bags were lost during the census. Two hundred ungerminated seeds remained in each bag were recollected and each seed was dissected. If an embryo of a seed was white, the seed was regarded as viable. If an albumen of a seed was rotten, the seed was regarded as died.

Data analysis

The relationships between environmental factors were analyzed by Pearson correlation. Numbers of germinated seedlings per bag were analyzed using generalized liner mix model (GLMM) with Poisson error distribution. In this model, the response variable was number of germinated seedlings per bag; the explanatory variables were distance from the river flow or canopy openness. The random factor was line transects.

Numbers of viable seeds per bag were analyzed using GLMM with Poisson error distribution. In this model, the response variable was number of viable seeds per bag; the explanatory variables were distance from the river flow or canopy openness. The random factor was line transects.

Numbers of seedlings or saplings in a quadrat were analyzed using GLMM with Poisson error distribution. In this model, the response variable was number of seedlings/saplings in a quadrat; the explanatory variables were distance from the river flow, canopy openness and their interaction. The random factor was line transects.

All analyses were performed with the statistical software R version 3.4.2. The lme4 package was used to calculate GLMMs. To determine the effects of explanatory variables, I used likelihood ratio test to compare models with and without the explanatory variables using Type Ⅱ ANOVA with χ^2 test.

Results

Microenvironments in quadrats

The mean distance of quadrat from the river flow was 25.22 m. The closest quadrat was 2.0 m away from the river flow and the farthest one was 61.9 m. The mean height of quadrat above the river flow was 8.72 m. The maximum height of quadrat above the river flow was 33.01 m, and the minimum was 0.22 m. Eight quadrats near the flow were flooded after

heavy rain caused by typhoon No. 13 in August 2018 (Fig. 2-1 A). The flooded quadrats were stimulated lower than the 1.38 m height above the river flow. Canopy openness correlated negatively with the distance from the river flow (Fig. 2-1 B). The maximum value of canopy openness was 18.1 % and the minimum was 0.7 %. The mean canopy openness was 5.69 %.

Seed bag experiment

The total number of germinated seedlings was 176 and more than 50 % of seedlings appeared in June (Fig. 2-2). The mean number of germinated seedlings per bag was 4.76. The maximum number of geminated seedlings per bag was 13, and the minimum was 0. Number of germinated seedlings was not significantly affected by the distance from the flow and canopy openness (Table 2-1; Fig. 2-3).

The total number of viable seeds was 317. The mean of viable seeds per bag was 9.61. The maximum number of viable seeds per bag was 29 and the minimum was 1. Number of viable seeds was not significantly affected by distances from the flow (Table 2-2; Fig. 2-4 A). Number of viable seeds was significantly affected by canopy openness (Table 2-2; Fig. 2-4 B).

Number of *P. hispida* in a quadrat

Forty-nine seedlings and/or saplings appeared in 16 of 76 quadrats and the largest number of plants in a quadrat was 11. Number of seedlings and/or saplings was significantly affected by canopy openness but not affected by the distance from the flow (Table 2-3; Fig. 2-5)

Discussion

The microenvironment in a mountain valley

Environmental conditions in the quadrats changed from the river flow to the ridge.

The floods damaged the areas ca. 1.5 m above the river flow and degrees of damage gradually decreased from the river flow to ridge. Hence, only eight quadrats near the river flow were flooded after the heavy rain caused by the typhoon (Fig. 2-1 A). Similar phenomena were also reported in other riparian forests (Sakio, 1997; Kaneko, Takada, & Kawano, 1999; Kaneko & Kawano, 2002). Light environments also changed along with distances from the river in a mountain valley. Distances from the flow correlated negatively with canopy openness (Fig. 2-1 B), which suggested that bright spots concentrated near the river flow whereas shaded spots near ridges below forests. Therefore, environmental conditions in the quadrats had a gradient from the river flow to the ridge.

Seed bag experiment

More than 50 % of germinated seedlings appeared in June (Fig. 2-2), which suggested that seeds of *P. hispida* germinated before the rainy season. Although *P. hispida* is said to be disturbance-dependent species, germination would not be necessarily limited after disturbances but seasonally dependent.

Exceptionally low percentage of germination per bag is inconsistent with my prediction. The percentage of germination in this experiment was lower than the previous study in which laboratory germination tests showed about 20% in average (Nishio et al., 2009). The result of laboratory germination tests would overestimate germination under natural conditions because this experiment used vermiculite that was suitable for germination of *P. hispida* (personal observation). In addition, seeds of *P. hispida* are dispersed on various soil substrata such as sand, gravel, rock, soil, and decay trees, and soil substrates often affect establishment of tree species (Christie & Armesto, 2003; Kubo et al., 2004; Masaki et al., 2007; Yuen & Seagle, 2013). Therefore, percentage of germinations under natural conditions would be lower than this

experiment.

The effect of environmental factors on the germination of *P. hispida* was restricted. Distances from the river flow significantly but slightly affected numbers of germinations per bag (Fig. 2-3 A), and the canopy openness did not significantly affect numbers of germinations per bag (Fig. 2-3 B). There would be two reasons. First, *P. hispida* would be able to germinate under various light conditions. Indeed, germination was observed even under 5% or over 10% of canopy openness (Fig. 2-3 B). Second, we did not know germination under very high canopy openness. Indeed, only one quadrat exhibited over 15% of canopy openness. The lack of strong environmental preference and low percentage of germination of *P. hispida* would weaken environmental gradients of seed germination.

Viable seeds were observed in all seed bags, which suggests seed dormancy of *P. hispida* and germination two years later after the seed dispersal. Because *P. hispida* is a pioneer tree, dormant seeds would escape various and repeated disturbances that kill seedlings in a mountain valley. Some disturbance-dependent species distributed in a mountain valley are known to have dormancy while many of the late successional species do not have dormancy. *Euptelea polyandra* is mainly distributed in a frequently disturbed area (Sakai & Ohsawa, 1993, 1994) and has dormant seeds (Kubo et al., 2008). Contrarily, *Fraxinus platypoda* and *Pterocarya rhoifolia* dominate stable stands (Sakio, 1997; Sakio et al., 2002) and their seeds do not have dormancy (Kubo et al., 2008).

Canopy openness negatively correlated with numbers of dormant seeds (Fig. 2-4 B), which suggests that the seeds dispersed to shade places would be dormant. A pioneer tree, *P. hispida*, would be a sun plant and avoid severe shading.

Seedlings/saplings in the quadrats

Forty-nine individuals appeared in 16 of 76 quadrats, which suggests that amounts of dispersed seeds were limited in the quadrats. Numbers of germination did not increase under a specific environmental condition (Fig. 2-4). The result suggested seeds of *P. hispida* would germinate under various environment in a mountain valley. Although germination of *P. hispida* do not need specific environmental conditions, the percentage of germination was very low. Low percentage of germinations indicated that establishment of a single seedling needed a large amounts of seed dispersal. In addition, germinated seeds were subjected to high mortality because, seed-to-seedling stages are most sensitive to abiotic environments (Harper, 1977). Canopy openness would have worked as environmental filtering because of the significant effects of canopy openness on plant numbers in a quadrat (Fig. 2-5 B). Therefore, the small number of plants in the quadrats reflected the small amounts of seed dispersal and environmental filtering.

Table 2-1 The effects of distances from the river flow (D) and canopy openness (C) on number of germinations per bag.

Source	Estimate	Std.Error	Chisq	Df	Pr
D	0.010	0.005	3.90	1	0.048
C	0.013	0.022	0.31	1	0.577

Table 2-2 The effects of distances from the river flow (D) and canopy openness (C) on the number of viable seeds per bag.

Source	Estimate	Std.Error	Chisq	Df	Pr
D	-0.003	0.006	0.25	1	0.616
C	-0.134	0.027	27.54	1	<0.001

Table 2-3 The effects of distances from the river flow (D), canopy openness (C) and their interaction (D:C) on number of plants in a quadrat.

Source	Estimate	Std.Error	Chisq	Df	Pr
D	-0.022	0.992	0.00	1	0.957
C	0.182	0.085	20.22	1	<0.001
D:C	0.004	0.005	0.61	1	0.434

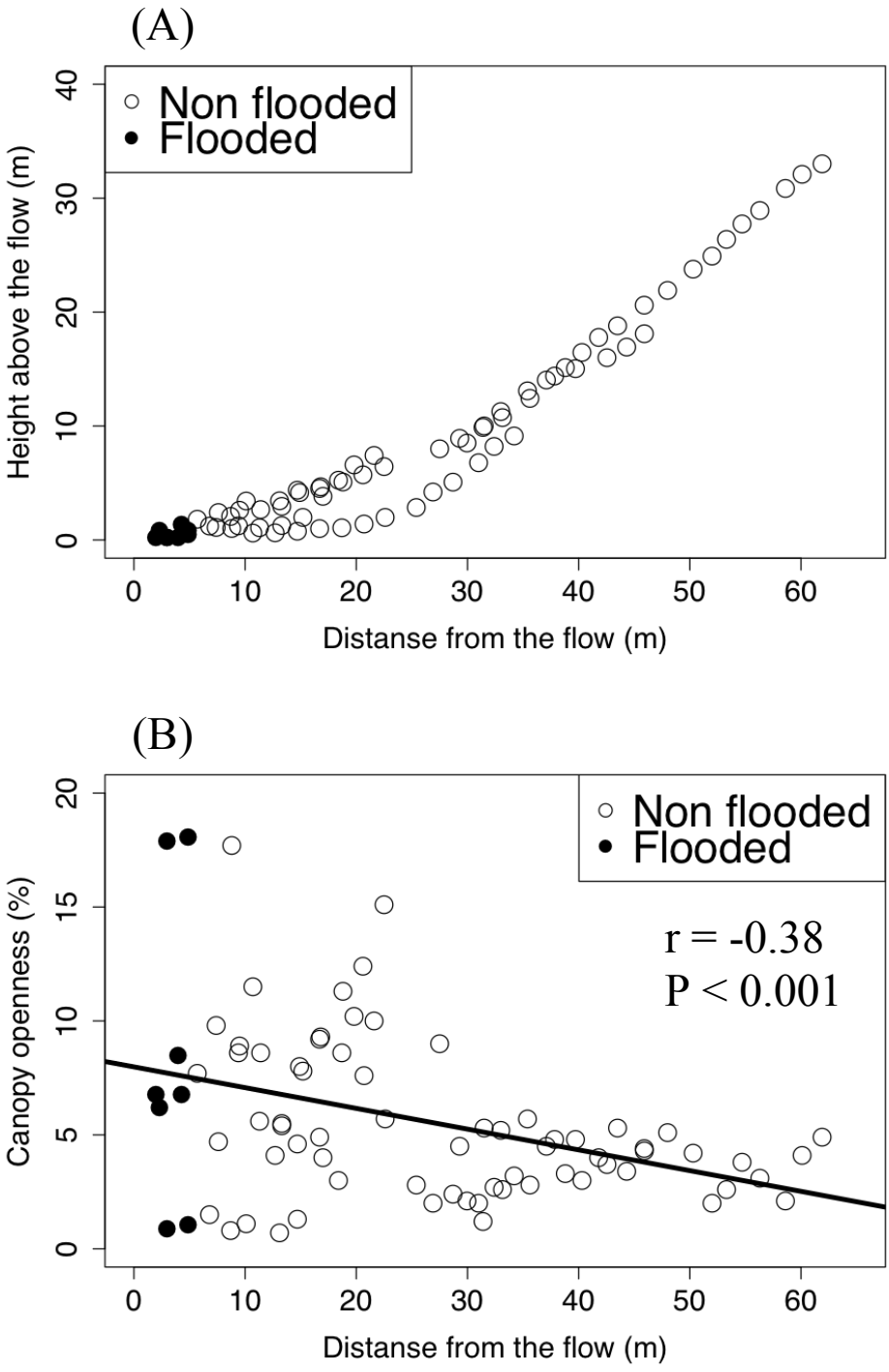

Fig. 2-1 The relationship between distance from the flow and (A) height above the flow and (B) canopy openness. A closed dot represents a quadrat which was flooded after the typhoon. An open dot represents a quadrat which was not flooded after the typhoon.

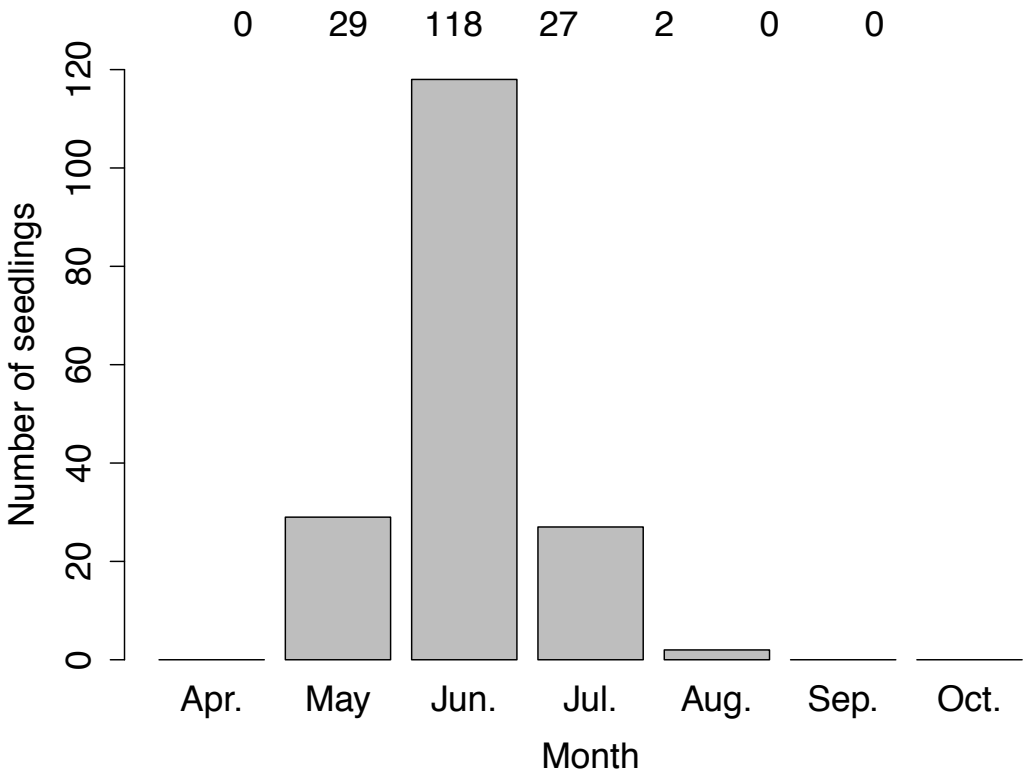

Fig. 2-2 Number of seedlings in all bags. Horizontal axis shows the month. Vertical axis shows germinated seedlings number. Numbers at the top of a bar shows number of germinated seedlings in each month.

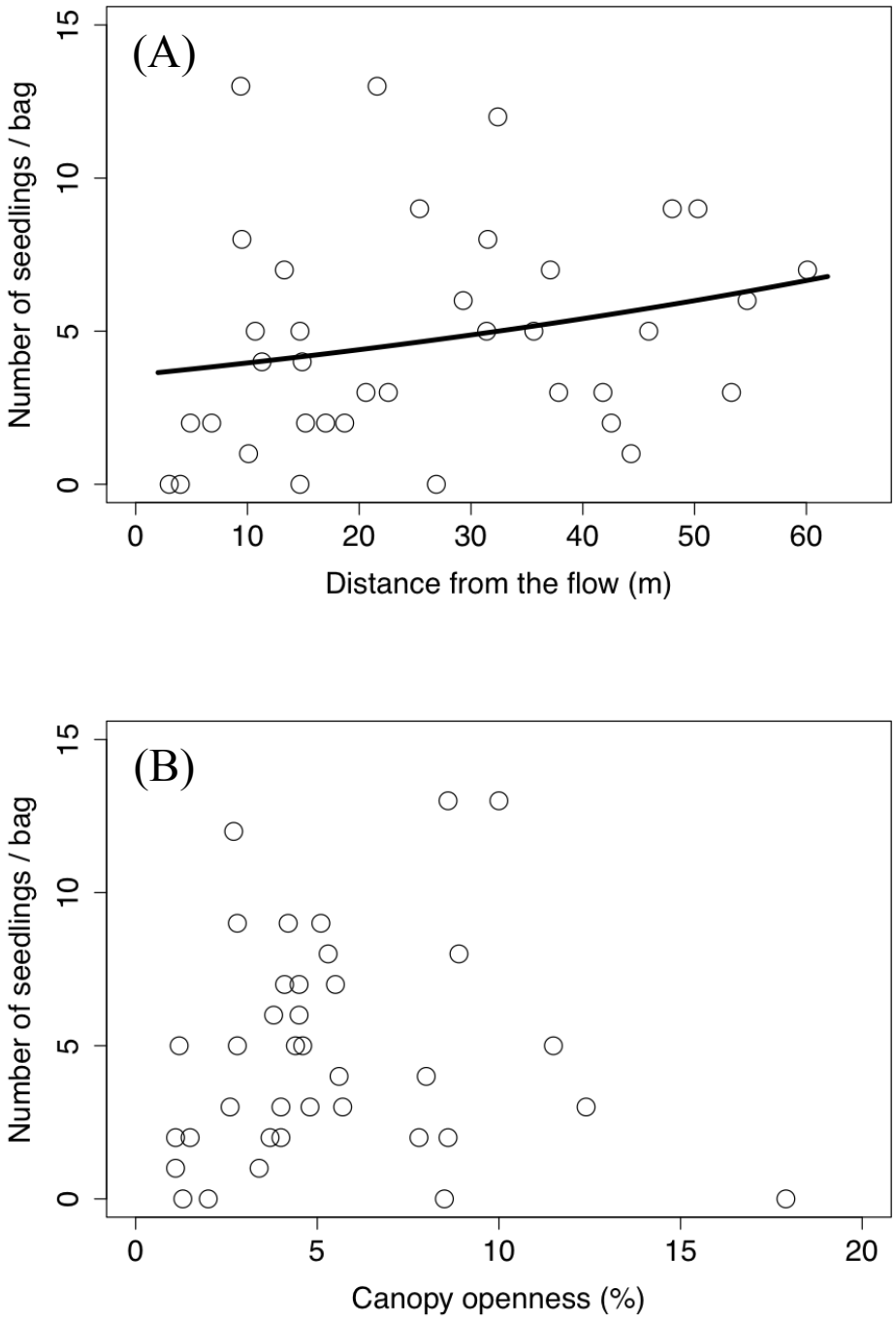

Fig. 2-3 The relationships between (A) distance from the flow or (B) canopy openness and number of seedlings per bag. The regression line was estimated by GLMM.

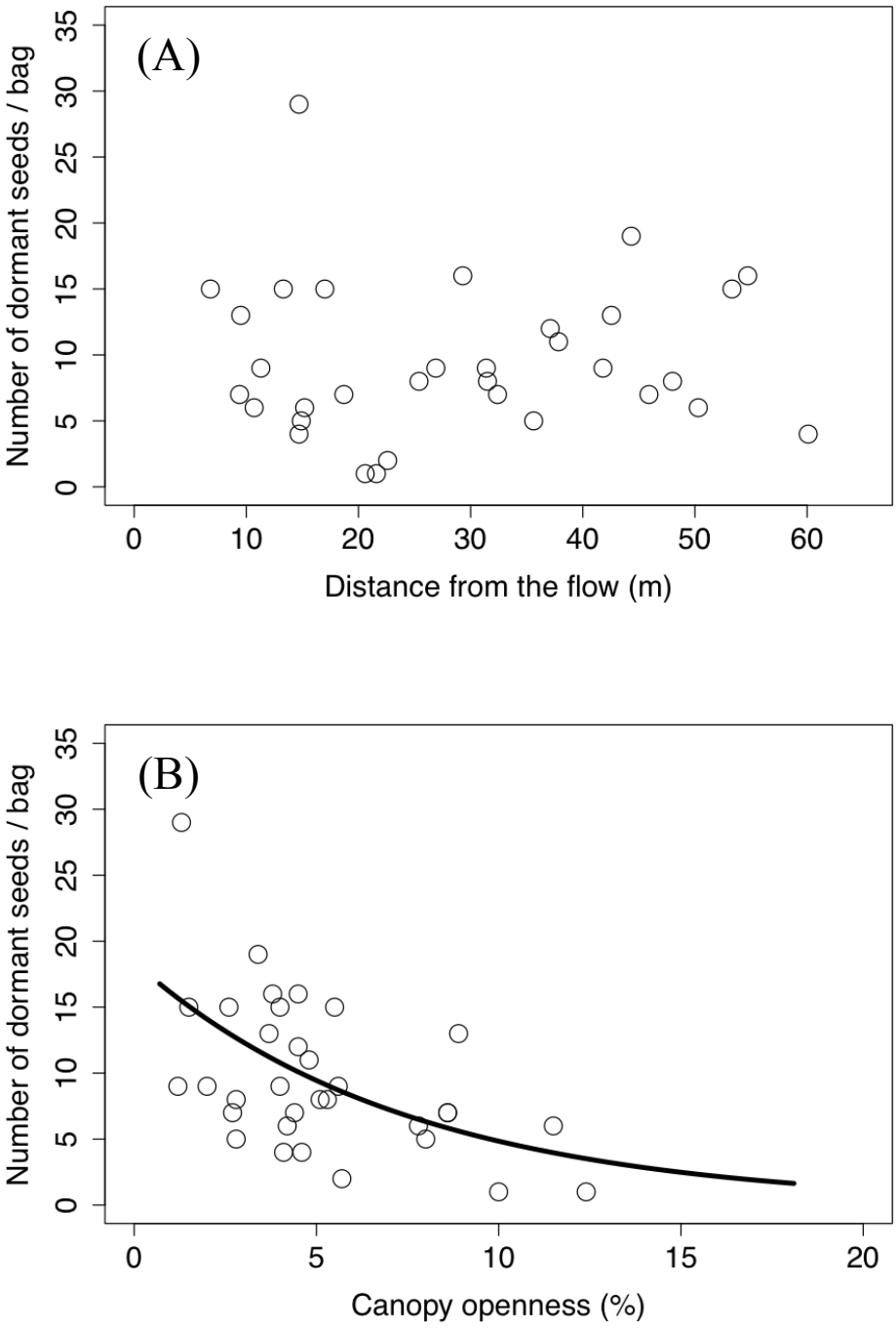

Fig. 2-4 The relationships between (A) distance from the flow or (B) canopy openness and number of dormant seeds per bag. The regression line was estimated by GLMM.

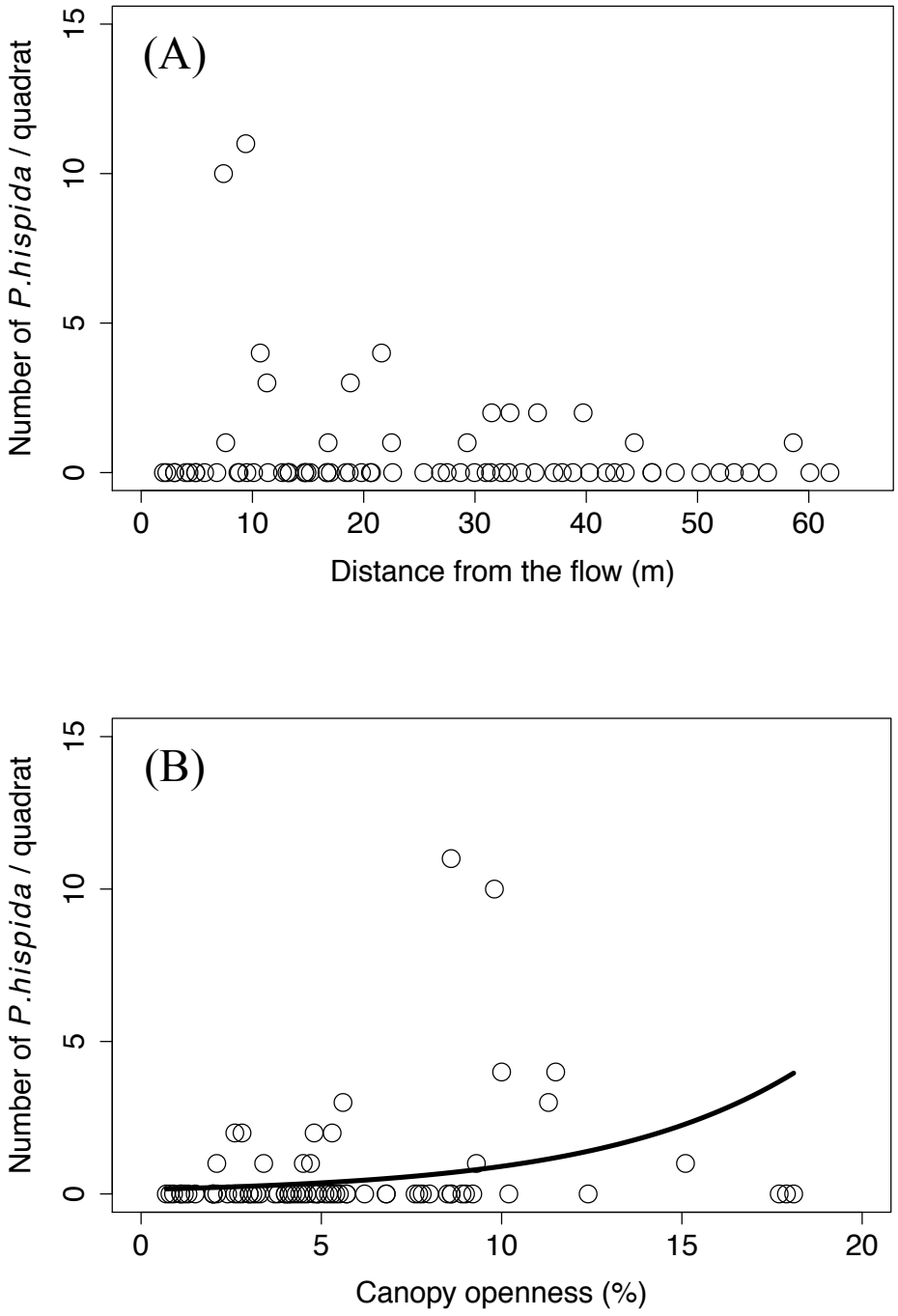

Fig. 2-5 The relationships between (A) distance from the flow or (B) canopy openness and number of *P. hispida* per quadrat. The regression line was estimated by GLMM.

Chapter 3

Effects of temporally heterogeneous flooding on survival and growth of current-year seedlings of a pioneer tree *Pterostyrax hispida*, in a mountain valley

Introduction

Disturbance regime is complex processes and affects regeneration of trees in a mountain valley. Gap formation by canopy disturbance and destruction of understorey vegetation by fluvial processes, such as floods or debris flows, often occur in riparian forests (Sakio, 1997; Suzuki et al., 2002). Flood physiologically damage trees. The lack of oxygen affects vital physiological and metabolic pathways and causes reduction of growth or even death of a plant. In general, flood tolerance of trees is evaluated in terms of growth response, level of injury and survival in relation to flooding level and duration (Kozlowski, 1997). Soil flooding reduces root growth and induces extensive root decay (Kozlowski, 1997, 2000). Rapid depression of leaf emergence and increases in leaf abscission were observed in responses of trees to floods (Terazawa & Kikuzawa 1994). Flooding near river flow restricts survival and growth of plants.

Responses of plants to floods have been studied all over the world because plant niches can be attributed to species-specific ranges of soil water conditions (Silvertown, Araya, & Gowing, 2015). In Europe, Vervuren, Blom, & de Kroon (2003) analyzed the range and variability of flooding components in the River Rhine and quantified their effects on the ability of riparian herbs to survive periods of submergence under experimental conditions for 8 weeks. Van Eck et al.(2004) examined survival and growth responses of grassland species of river

floodplains to total submergence during summer. In north America, twelve tree species common in Everglades tree islands were subjected to three hydrologic regimes under controlled conditions for 25 weeks and assessed for growth and physiological responses (Jones et al., 2006). In central America, Lopez & Kursar (2003) evaluated flood tolerance in tropical tree species for 90 days where flooded forests can experience flooding of up to 10 months. Many studies have examined the effects of flooding or submergence for long period on plant growth.

The effects of floods on plant growth have been also studied in Japan. Sakio (2005) investigated the effects of flooding on seedling growth of woody riparian species with different habitats of the cool-temperate forest for over 7 months. Higa et al. (2012) studied response and tolerance of current-year seedlings of two *Salix* and three Ulmaceae species from warm-temperate region to submergence treatments for 1, 2, 4, and 8 weeks. However, flood period in a mountain region in Japan is shorter than those in the experiments. River flow rates in mountainous areas in Japan suddenly changes because even a large river has short flow path and a steep gradient (Tani, 2016). Furthermore, repeated heavy rain due to rainy seasons and typhoons vary daily precipitation. Hence, floods in a mountain valley in Japan repeats but strikes shortly. Although flood periods in a mountain valley in Japan are more temporally heterogeneous than experimental conditions, the effects of temporally heterogeneous floods on trees in a mountain valley remain unclear (but see Nakai, Yurugi, & Kisanuki, 2010; Nakai & Kisanuki, 2011).

A pioneer tree, *Pterostyrax hispida*, is one of the common species in a mountain valley (Oohashi et al., 2017). They often distributed in disturbed areas such as forest-road verges and the riversides. Number of *P. hispida* is increasing in mountainous areas in Kanto region (Ninomiya & Furubayashi, 2003) although most of the palatable plants disappeared due to the severe grazing of sika deer (Sakio et al., 2013; Takatsuki, 2009). Seedings or saplings of *P.*

hispida would facilitate recruitment of other palatable plants that have been severely grazed by sika deer (Ishida et al., 2008; Tamura, 2014; Tamura et al., 2016). Therefore, environmental conditions of safe sites for *P. hispida* seedlings, and responses of seedlings to floods should be studied.

I evaluated the effects of temporally heterogeneous floods on current-year seedlings of *P. hispida*. I hypothesized *P. hispida* would be subject to frequent floods and drought on riverbanks in a mountain valley and survive the conditions. The following questions were tested, (1) Do floods affect survival of *P. hispida*? (2) Do floods affect leaf dynamics? (3) Do floods change plant growth?

Materials and Methods

Material plants and their natural habitats

In October 2016, seeds of *P. hispida* were collected from two mother trees in a population in the University of Tokyo Chichibu Forest and preserved under wet and cold conditions at 4 ℃.

A mother tree was located near the River Oochi in the University of Tokyo Chichibu Forest, and many seedlings or saplings of *P. hispida* were occurred in riverbanks ca. 50 cm above the river flow (personal observation). If the water level reaches 50 cm above the normal water level, some seedlings of *P. hispida* would be submerged. Therefore, the flooding period in which seedlings or saplings of *P. hispida* are submerged is defined as the period of the water level beyond 50 cm above the normal level. Because the water level of the River Oochi is not measured, I used the data of the water level of the River Oobora (Ministry of Land, Infrastructure, Transport and Tourism) near the University of Tokyo Chichibu Forest. The river water level rose over 50 cm above the normal level for 23 times from June to October between

2012 and 2016. The average period was 2.52 days and the longest period was 8 days (Fig. 3-1).

Experimental design

The experiment was carried out from April to October 2017 in a plastic film greenhouse under natural sunlight in the experimental garden of Tokyo Metropolitan University (Hachioji, Tokyo; 35 ° 379N, 139 ° 239E). The experiment had a two-way factorial randomized block design with eight replications. The factors were mother trees and flood treatment. There were two mother trees: A and B. Flood treatment included four levels; submerged a pot for 2 days and emerged the pot for 2 days in the 2 days flood treatment, submerged a pot for 4 days and emerged the pot for 4 days in the 4 days flood treatment, submerged a pot for 8 days and emerged the pot for 8 days in the 8 days flood treatment and the control treatment in which a pot was unsubmerged and watered every 2 days. The total period of flood was the same for all flood treatments except for the control treatment. During a flood period, water was changed every 2 days. Flood period was determined by the data of the water level of the River Oobora, and the maximum period of flood was determined as 8 days in this experiment. Seeds of *P. hispida* were sown in plastic cell trays from 27 to 29 April and 64 seedlings were obtained. The seedlings were transplanted into a plastic pot with 31 cm in diameter and 32 cm in height tall on June 30. Pots were filled with 12 L of a mix of granular red cray and humus (ratio 3:2). For the 20 days after transplanting, all pots were watered sufficiently every 2 days. On the 20th day after transplantation, we begun the flood treatment under which a pot was submerged in a 12 litters plastic bucket from July 20 to October 24 for 96 days. The water level was the same as soil surface in a pot and kept during a flood period.

To determine the response of current-year seedlings of *P. hispida* to temporally heterogeneous flood, survival of plants was recorded every 2 days and number of leaves of each

plant was recorded every 16 days.

Plants were harvested on the 96 days after the beginning of flood treatment at which all three flood periods were simultaneously terminated. Harvested plants were divided into leaves, stems and roots. They were then dried at 70 ℃ for 3 days and weighted. Plant biomass was defined as the sum of leaves, stem and root of each plant.

Data analysis

Mortality of plants were analyzed by generalized linear mixed model (GLMM) with binomial error distribution. In this model, the response variable was plant mortalities; the explanatory variables were flooding treatment, mother tree and their interaction. The random factor was block.

The number of leaves of each plant was analyzed by GLMM with Poisson distribution. In this model, the response variable was the number of leaves of each plant; the explanatory variables were flood treatment, mother tree and their interaction. The random factor was block.

The plant biomass was analyzed by GLMM with gamma error distribution. In this model, the response variable was belowground or aboveground biomass; the explanatory variables were flooding treatment, mother tree and their interaction. The random factor was block.

All analyses were performed with the statistical software R version 3.4.2. The lme4 package was used to calculate GLMMs. To determine the effects of explanatory variables, I used likelihood ratio test to compare models with and without the explanatory variables by using Type Ⅱ ANOVA with χ^2 test.

Results

Plant survival

Plant survival was not significantly affected by flood treatment and mother trees at 16 days, 32 days, 48 days, 64 days, 80 days and 96 days (Table 3-1). Numbers of the survived plants by the end of experiment were 7 in the control treatment, 5 in the 2 days flood treatment, 7 in the 4 days flood treatment and 7 in the 8 days flood treatment for the mother tree A, and 7 in the control treatment, 7 in the 2 days flood treatment, 8 in the 4 days flood treatment, 5 in the 8 days flooding treatment for mother tree B (Fig. 3-2).

Number of leaves

At the beginning of treatments, numbers of leaves did not differ between the treatments. Numbers of leaves were not significantly affected by flood treatment and mother trees at 16 days, 32 days, 48 days, 64 days, 80 days and 96 days after the beginning of the treatments (Table 3-2; Fig. 3-3). In any treatments, numbers of leaves once decreased, and increased (Fig. 3-3).

Biomass

Aboveground biomass was significantly affected by the flood treatments but not by the mother tree (Table 3-3; Fig. 3-4). Aboveground biomass of the 4 days flood treatment was the largest of the mother tree A (Fig. 3-4), and that of the control treatment was the largest of the mother tree B (Fig. 3-4). Belowground biomass was significantly affected by the flood treatments and the mother tree (Table 3; Fig. 3-4). Belowground biomass of the control treatment was the largest of both mother trees (Fig. 3-4).

Discussion

A flood risen 50 cm above the normal level did not last beyond 8 days in the River Oobora between 2012 and 2016 except for one case (Fig. 3-1). Records on floods in this river showed that floods for a few days frequently occurred especially in rainy seasons or typhoon seasons in this mountain valley in Japan. Moreover, steep gradients of mountains accelerate river flow in Japan (Tani, 2016). Thus, *P. hispida* in riverbanks in a mountain valley would be repeatedly subjected to floods for short periods.

The effects of temporally heterogeneous flood on the survival was not significant (Fig. 3-3, Table 3-1), which suggests that current-year seedlings of *P. hispida* would survive for ordinally floods. In this experiment, flood terms were less than 8 days because a flood seldom lasted beyond 8 days in Chichibu mountains and short term floods did not cause crucial effects on current-year seedlings of *P.hispida* on the contrary to my prediction. The total submerge period of this experiment was 48 days that were longer than one month. Although first-year seedlings of *P. hispida* began to die within one month in continuous flood (Sakio, 2005), the severe conditions of this experiment were not lethal to seedlings of *P. hispida* at least during the experimental periods. Hence, repeated interval periods, thus under draining between floods would be important for seedlings of *P. hispida* to survive in riverbanks in a mountain valley.

The effects of temporally heterogeneous floods on numbers of leaves were not significant during the experimental period (Table. 3-2, Fig. 3-3), which was inconsistent with previous studies. In previous studies, species with weak tolerance to floods are often sensitive to submergence. Leaf dynamics of seedlings of *P. hispida* flooded for 7 months differ from those of flood tolerant species (Sakio, 2005). Submerged seedlings of *P. hispida,* begins to lose leaves 2 months after the beginning of experiment, while those of flood-tolerant species, *Fraxinus platypoda* do not drop their leaves (Sakio, 2005). The suppression of shoot elongation and leaf production after flood treatments is observed in *Aphananthe aspera* that is less tolerant to

submergence compared to the flood-tolerant species, *Salix* species (Higa et al., 2012). Seedlings of *Betula platyphylla var. japonica* cannot invade swampy sites that often suffer from frequent soil inundation because the seedlings rapidly lose their leaves in response to floods (Terazawa & Kikuzawa, 1994). In this experiment, the leaf dynamics of *P. hispida* (Fig. 3-3) differed from those of flood-intolerant species, which suggested types of floods would cause diverse responses to submergence especially in species with weak or moderate flood tolerance. Hence, the effects of temporally heterogeneous floods on leaf dynamics were limited.

Temporally heterogeneous flood significantly decreased the biomass increment (Table 3-3, Fig. 3-4), which suggested that short-term floods reduced plant growth as well as long-term floods. Indeed, growth of first-year seedlings of *P. hispida* is inhibited by the flood for 7 months (Sakio, 2005). However, the effects of water deficiency on *P. hispida* is still unclear. Present results did not determine which of flood or drought was more influence on the growth. Repetitive floods and droughts do not diminish stresses caused by flood or drought but successive repetition of submergence and dry periods additively reduces the growth of damaged roots of *Salix gracilistyla* (Nakai et al., 2010). In the case of *P. hispida*, hence the effects of short-term floods and droughts on plant growth would be additive.

This study quantitatively showed that temporally heterogeneous floods with reasonable terms significantly reduced plant growth but caused no mortality or no leaf abscission. These results were inconsistent with the results of the pot experiment to test effects of long-term floods (Higa et al., 2012; Sakio, 2005), which suggested that the effects of short-term floods differed from long-term floods. Many studies on the effects of long lasting floods on plants have been conducted (Lopez & Kursar, 2003; Vervuren et al., 2003; Van Eck et al., 2004; Jones et al., 2006). However, frequent and short-term floods are the norm in riverbanks in a mountain valley especially in Japan. Present study exhibited that seedlings of *P.*

hispida were able to survive but their growth was retarded under frequent and short-term flooding in a mountain valley.

Table 3-1 Effects of flooding (F), mother tree (M) and their interaction (F:M) on the number of survivors each day after the beginning of the flood treatment.

Day	Source	Chisq	Df	Pr(>Chisq)
17	F	0.000	3	1.000
	M	0.000	1	1.000
	F:M	0.000	3	1.000
33	F	0.000	3	1.000
	M	0.000	1	1.000
	F:M	0.000	3	1.000
49	F	0.583	3	0.900
	M	0.000	1	1.000
	F:M	0.000	3	1.000
65	F	0.914	3	0.822
	M	0.095	1	0.758
	F:M	1.531	3	0.675
81	F	1.659	3	0.646
	M	0.000	1	1.000
	F:M	2.457	3	0.483
97	F	1.659	3	0.646
	M	0.000	1	1.000
	F:M	2.457	3	0.483

Table 3-2 Effects of flooding (F), mother tree (M) and their interaction (F:M) on the number of leaves each day after the beginning of the flood treatment.

Day	Source	Chisq	Df	Pr(>Chisq)
1	F	0.107	3	0.991
	M	1.662	1	0.197
	F:M	0.109	3	0.991
17	F	3.833	3	0.280
	M	0.012	1	0.914
	F:M	0.238	3	0.971
35	F	1.606	3	0.658
	M	1.991	1	0.158
	F:M	1.463	3	0.691
49	F	1.606	3	0.658
	M	1.991	1	0.158
	F:M	1.463	3	0.691
67	F	2.492	3	0.477
	M	0.529	1	0.467
	F:M	0.089	3	0.993
82	F	2.201	3	0.532
	M	1.325	1	0.250
	F:M	0.432	3	0.934
97	F	3.259	3	0.353
	M	0.770	1	0.380
	F:M	0.752	3	0.861

Table 3-3 Effects of flooding (F), mother tree (M) and their interaction (F:M) on aboveground and belowground biomass.

Source	Chisq	Df	Pr(>Chisq)
Aboveground			
F	13.694	3	0.003
M	4.593	1	0.032
F:M	6.326	3	0.097
Belowground			
F	24.948	3	<0.001
M	1.768	1	0.184
F:M	4.140	3	0.247

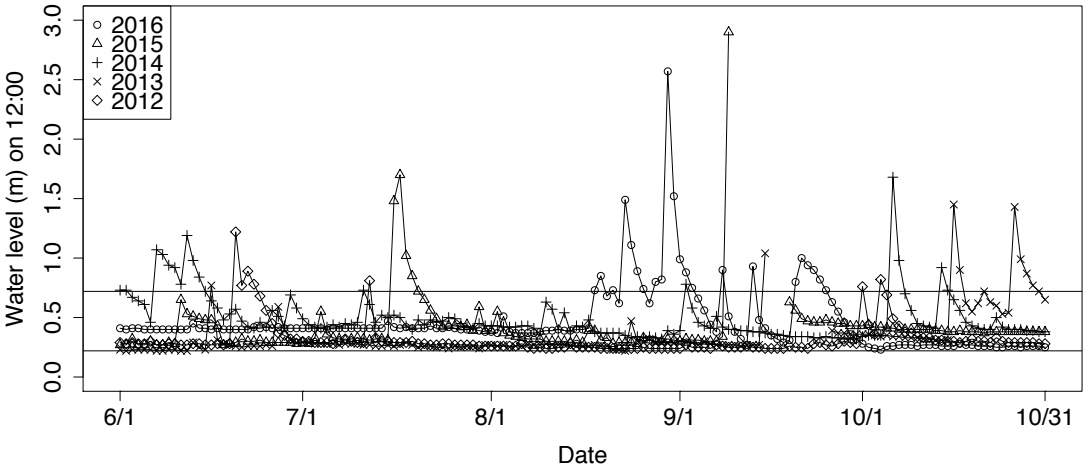

Fig. 3-1 Change in the water level of River Oobora. Horizontal axis indicates the date from 6/1 to 10/31. Vertical axis indicates the water level at 12:00 on each day. The minimum water level was 0.22 m between 2012 and 2016. The water level beyond 0.5 m above the minimum water level was 0.77 m.

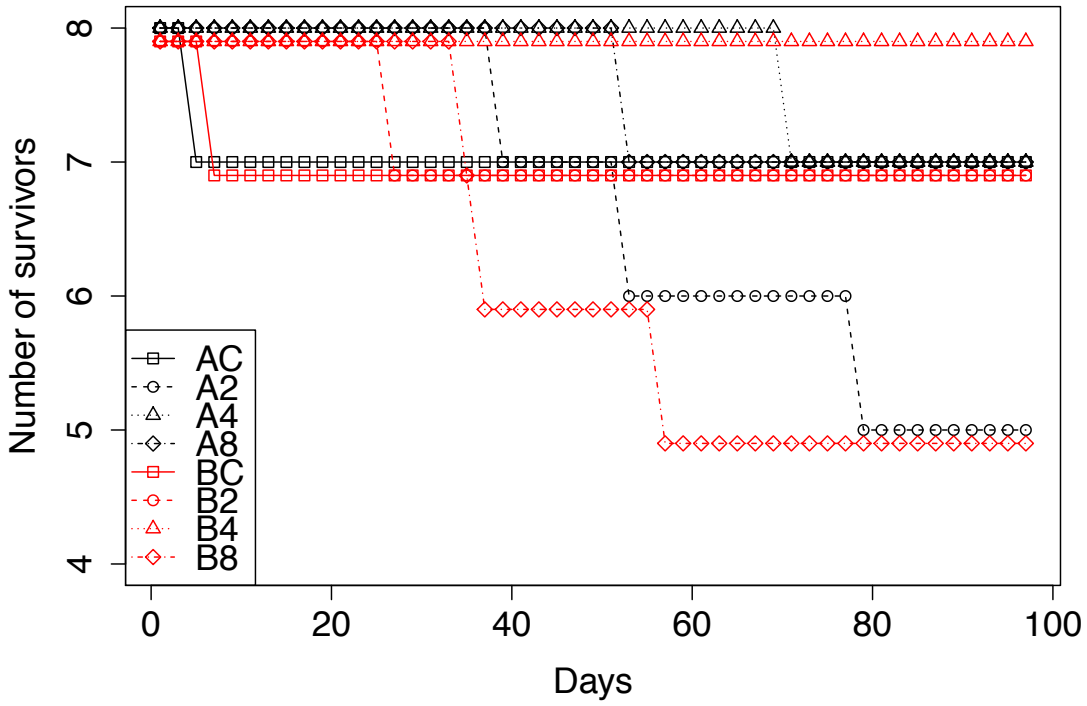

Fig. 3-2 Changing the number of survivors in each treatment. Horizontal axis indicates the day after beginning of the flood treatment. Vertical axis indicates the number of survivors in each treatment. Black symbols show the mother tree A. Red symbols show mother tree B.

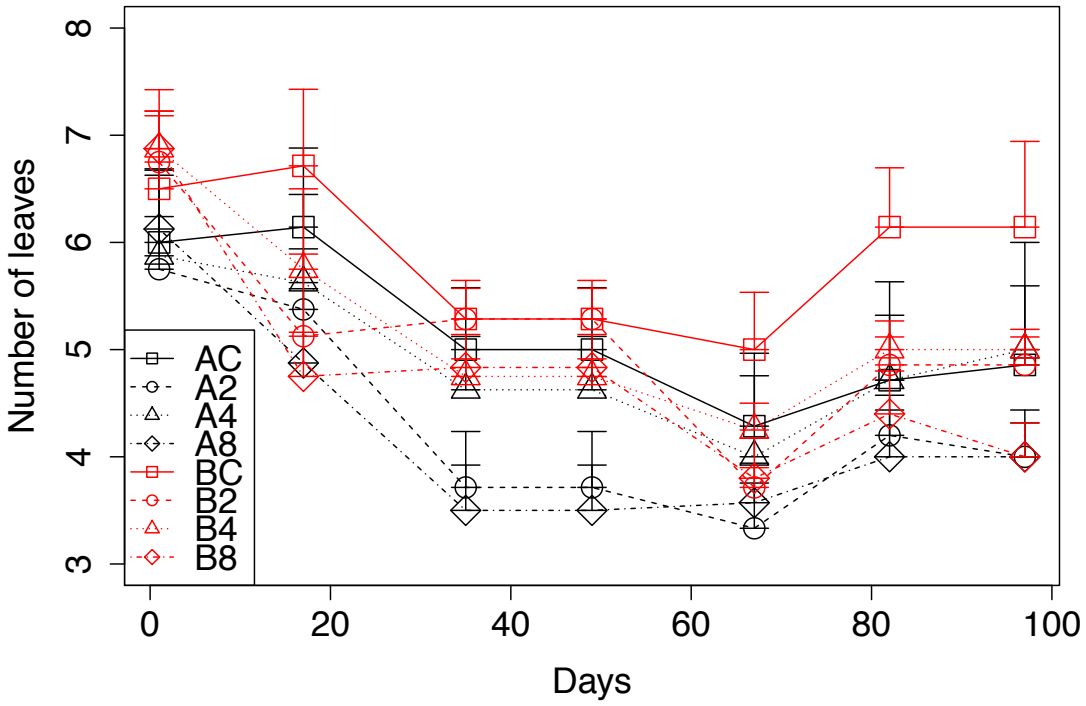

Fig. 3-3 Changing mean number of leaves in a seedling. Horizontal axis indicates the day after beginning of the flood treatment. Vertical axis indicates mean number of leaves in each treatment. Blank symbols show the mother tree A. Solid symbols show mother tree B. Vertical bars indicates standard error.

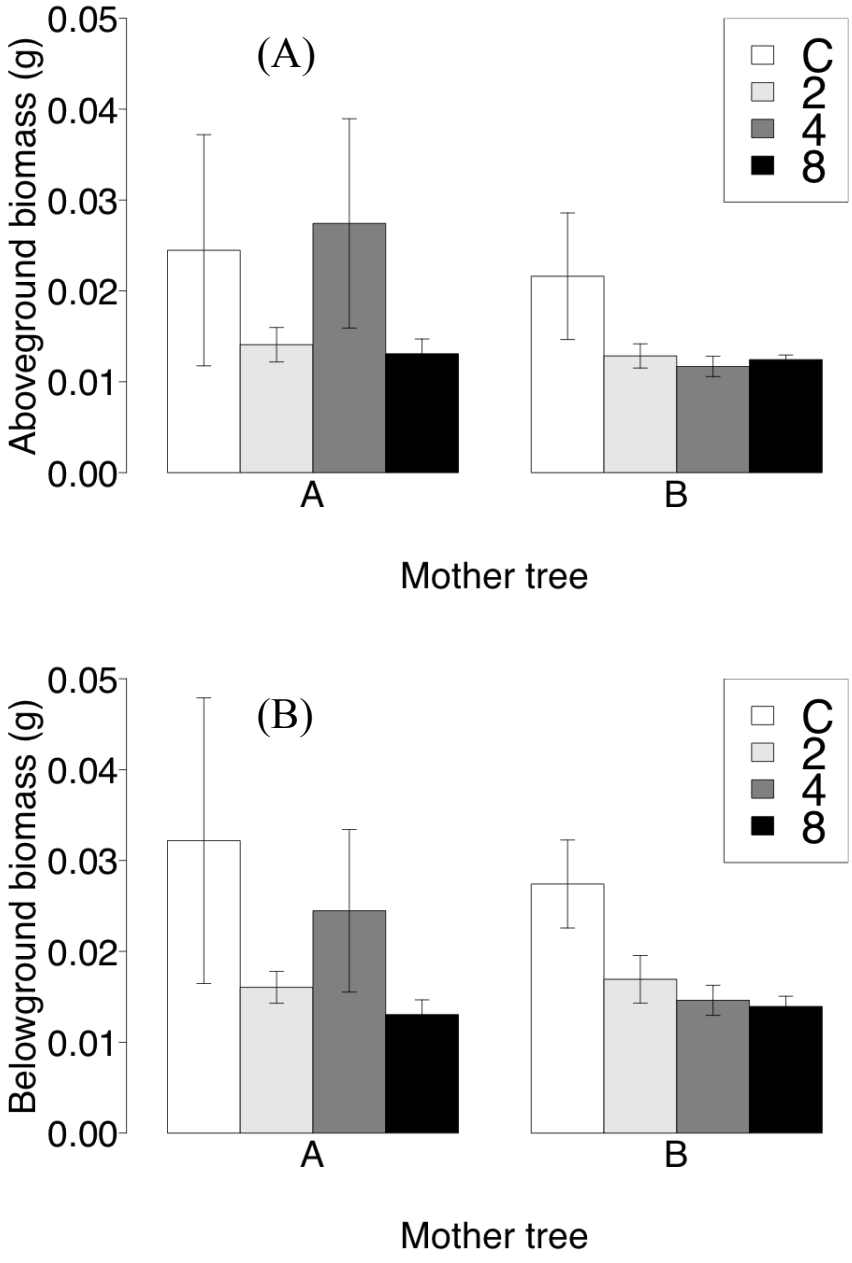

Fig. 3-4 Mean biomass (± SE) of (A) aboveground biomass and (B) belowground biomass in the different flood treatments (C, 2, 4 and 8 means control treatment, 2 days flood treatment, 4 days flood treatment and 8 days flood treatment, respectively)

Chapter 4

Effects of pot diameters and soil-particle size with the same pot-volume on the survival and growth of current-year seedlings of *Pterostyrax hispida* and *Euptelea polyandra*

Introduction

A mountain valley often develops specific vegetation patterns due to the various and frequent disturbances. Floods or debris flow destroy canopies and understories of riparian forests (Sakio, 1997; Suzuki et al., 2002; Vervuren, Blom, & de Kroon, 2003) and differences in disturbances bring about heterogeneous microsite structure in riparian areas (Nakamura, Yajima, & Kikuchi, 1997) and various microtopography. These disturbances repeatedly occur with various intensities in riparian forests. Therefore, diverse disturbance regimes result in environmental heterogeneity, and the heterogeneous environments provide various niches for coexisting diverse species (Gregory, Swanson, McKee, & Cummins, 1991; Pollock, Naiman, & Hanley, 1998).

Seedling establishment of woody species is often affected by soil property in a mountain valley and each species has strong species-specific preference. Some studies reported that processes from seeds to seedlings are sensitive to substrate types (Christie & Armesto, 2003; Masaki et al., 2007; Yuen & Seagle, 2013). In a mountain valley, soil substrates are diverse and patchy. Sand or gravels are often accumulated on a riverside and mineral soils are scattered, while mature soil was distributed on hillslopes (Sakio, 1997). Therefore, seedling establishment of a certain species occurs only in a certain site which provides specific soil preferred by the species.

Differences in soil-surface shapes may also affect seedling establishment as well as soil substrata. First, soil moisture would influence seedling establishment and soil moisture is determined by soil-surface shapes and depths. If soil volume is the same, shallow soil with wide surface shape lose moisture faster than deep soil with narrow surface. Soil moisture and its temporal variability eventually affect plant biomass growth (Novoplansky & Goldberg, 2001; Hagiwara, Kachi, & Suzuki, 2008). Second, establishment of seedlings depends on soil properties because soil-depth determines root architecture. Root systems with many fine lateral roots may absorb effectively resources in rocky soils (Kohyama & Grubb, 1994). Species that can grow on thin soil may have changed root architecture depending on soil depth. There would be species-specific soil depth and soil-surface shape that allows survival and growth of seedlings of a certain species.

A garden experiment was carried out to examine the effects of soil-depths and soil-shape on the growth of woody seedlings through soil water content. In a garden experiment by using a pot, pot diameter and soil-particle size would change the water retention capacity. A pot with wide mouth would dry fast whereas a pot with narrow mouth would do slowly. Granular soil with large-sized particles would dry faster than that with small-sized particles. A pot diameter and soil particle sizes determine vary water retention capacity of the pot, and differences in pot diameters and the soil particle sizes water retention. The water retention changes soil water content of a pot, and consequently affect the plant growth. Thus, it is important to test the effects of soil-depth and soil-shape on the growth of woody seedlings.

Effects of soil-depth and soil-shape would depend on the species with preference to habitats. Both *P. hispuda* and *E. polyandra* are common species in a mountain valley (Oohashi et al., 2017) but their preference to habitats are different. *P. hispida* is distributed not only in a mountain valley but also on a ridge (Ninomiya & Furubayashi, 2003) and *E. polyandra* is

widely distributed on landslide slopes, at which soils are very thin (Sakai & Ohsawa, 1993, 1994). Different preferences to habitats might show different responses to the soil-shape and soil-depth. Species with different preference to habitats would respond differently to a certain combination of a pot diameter and a soil.

In this study, I investigated the effects pot diameters and soil particle sizes with same pot-volume on the survival and growth of current-year seedlings of *P. hispida* and *E. polyandra*. I test three hypotheses: (1) A pot diameter and soil particle size determine soil water content of the pot; a narrow pot with small-sized soil particles would retain more water in soil of a pot than a wide pot with large-sized soil particles even if the pots have the same volume. (2) Growth of seedlings is larger in a narrow pot with small-sized soil particles than in a wide pot with large-sized soil particles because a narrow pot with small-sized soil particles has high water availability. (3) The effects of pot diameters and soil-particle sizes on growth of seedlings are different between species because each species has species-specific preference to its habitat. I predict that the effects on *E. polyandra* is greater than that of *P. hispida* because seedlings of *E. polyandra* are more sensitive to water availability than those of *P. hispida*.

Materials and Methods

Material plants

In October 2017, seeds of *P. hispida* and *E. polyandra* were collected from natural populations in the University of Tokyo Chichibu Forest. The seeds of *P. hispida* were preserved under wet and cold conditions at 4 ℃ in the cold room. The seeds of *E. polyandra* were preserved under dry and cold conditions at 4 ℃ in the cold room. One hundred seeds of each species were dried at 70 ℃ for 3 days and weighted.

Experimental design

Growth experiment was carried out from April to November 2018 in a plastic film greenhouse under natural sunlight in the experimental garden of Tokyo Metropolitan University (Hachioji, Tokyo; 35 ° 379N, 139 ° 239E). The experiment had a three-way factorial randomized block design with fourteen replications. The factors were plant species, soil-particle size and pot diameter. There were two species: *P. hispida* and *E. polyandra*. There were two soil-particle sizes (mean ± SD, n = 100): small (7.16 mm ± 1.59) and large (18.07 mm ± 2.49). There were three types of pot diameters: narrow (10.5 cm diameter, 22.5 cm height), medium (16.5 cm diameter, 10.5 cm height) and wide (18.0 cm diameter, 9.0 cm height).

Seeds of *P. hispida* and *E. polyandra* were sown in plastic trays on 23 and 24 in April, 2018 and 84 seedlings of each species were obtained. The seedlings were transplanted into a polyester pot from 6 to 11 June. Pots were filled with 1.4 litters of granular red cray and the recommended amount (4 g) of slow-release fertilizers (Magamp K, 6 N:40 P:6 K:15 Mg; Hyponex Japan, Osaka, Japan). The pots were watered every day. To estimate water amounts of each soil, pot weights were measured before and after watering on 5 dates; 22 July, 13 August, 7 September, 25 September and 1 November. The difference in weights before and after watering was calculated.

Plants were harvested on November 2 and were divided into leaves, stems and roots. They were then dried at 70 ℃ for 3 days and weighted. Plant biomass was defined as the sum of leaves, stems and roots of each plant.

Data analysis

Differences in pot weights before and after watering were analyzed by generalized linear mixed model (GLMM) with gamma error distribution. In this model, the response

variable was the differences of pot weights; the explanatory variables were pot diameters, soil-particle size and their interactions. The random factor was measurement dates.

Numbers of survived plants were analyzed by generalized liner model (GLM) with binomial error distribution. In this model, the response variable was plant mortalities; the explanatory variables were plant species, pot diameters, soil-particle size and their interactions. I did not use GLMM because the model did not converge properly in this analysis.

The plant biomass was analyzed by GLMM with gamma error distribution. In this model, the response variable was the plant biomass; the explanatory variables were plant species, pot diameters, soil particle sizes and their interactions. The random factor was a block.

All analyses were performed with the statistical software R version 3.4.2. The lme4 package was used to calculate GLMMs. To determine the effects of explanatory variables, I used likelihood ratio test to compare models with and without the explanatory variables by Type Ⅱ ANOVA with χ^2 test.

Results

Seed weight

Dry weights of seeds of *P. hispida* and *E. polyandra* (mean ± s.d., n = 100) were 8.7 ± 2.28 mg and 2.2 ± 0.53 mg, respectively.

Difference in pot weights before and after watering

The difference in pot weights before and after watering was significantly affected by pot diameter, soil-particle size and their interaction (Table 4-1). Difference in pot weights of wide pots was the largest and that of narrow pots was the smallest (Fig. 4-1). Difference in pot weights with small soil-particles was larger than that with large soil-particles (Fig. 4-1).

Plant survival

Number of survival plants was significantly affected by species, pot diameter and soil-particle size (Table 4-2). For *P. hispida*, only 3 plants survived in narrow pots, 23 plants survived in medium pots and 20 plants survived in wide pots (Fig. 4-2 A). In total, 21 plants survived in pots filled with large-sized particles (Fig. 4-2 A). As for *E. polyandry*, 1 plant survived in a narrow pot, 4 plants in medium pots and 15 plants in wide pots (Fig. 4-2 B). In total, 4 plants survived in pots with large-sized particles (Fig. 4-2 B). Most seedlings were killed immediately after the rainy season that ended on 29 June 2018 in Tokyo.

Biomass

Plant biomass was significantly affected by pot diameter but not by the species and soil-particle size (Fig. 4-3, Table 4-3). Plant biomass of *P. hispida* was significantly affected by pot diameter (Table 4-3). Plant biomass was larger in wide pots than in narrow pots irrespective of soil-particle sizes in both species (Fig. 4-3).

Root architecture of seedlings

The length of root was different between pot diameters. Root of *P. hispida* did not reach the bottom of a narrow pot (Fig. 4-4) but did of a wide pot (Fig. 4-5).

Discussion

Pot diameter and soil-particle size affected the difference of pot weight before and after watering (Table 4-1). Pot weight difference was larger in wide pots than in narrow pots (Fig. 4-1) because rates of water loss would be higher in wide pots than in narrow pots, which is

consistent with my hypothesis. Differences in weights were larger in pots with soils of small-sized particles than those with soils of large-sized particles (Fig. 4-1), which is inconsistent with my hypothesis. Soils of large-sized particles are more likely to lose water because soils of large-sized particles include larger pores between particles. The pores would be filled with water after watering but cannot sustain water.

More plants died in narrow pots than in wide pots (Fig. 4-2), although wide pots were more likely to lose water (Fig. 4-1). The results that more plants died under slow-drying conditions are inconsistent with my prediction. The results strongly suggest pot water content would not be the primary reason to cause plant mortality in this experiment. Soil temperature in the core of a pot would be higher in a narrow pot than in a wide pot because the narrow pot has the smallest surface area exposed to the air compared to the wide pot (Poorter, Bühler, Van Dusschoten, Climent, & Postma, 2012). As soil water evaporates, it removes the heat from a pot. Therefore, a narrow pot would accumulate soil temperature, and hence the plants in narrow pots might be suffered lethal temperature. Actually, most of the mortality occurred on sunny days after rainy season. Therefore, soil surface shape would affect plant survival through soil conditions.

When soil-particle sizes were large, the mortality of *E. polyandra* was higher than that of *P. hispida* (Fig. 4-2), which suggested that the effects of soil-particle sizes on plant survival would differ depending on the species. One of the most obvious differences between these species was seed size. Seeds of *P. hipida* were heavier than those of *E. polyandra*. Large seedlings deriving from large seeds are more resistant to environmental stresses such as drought (Grubb, 1977). Thus, large seeds of *P. hispida* would allow their seedlings to expand their roots into favorable microenvironments (Gray & Spies, 1997). Present study suggests that soil-particle sizes did not determine seedling survival of *P. hispida* but affected the mortality of

E. polyandra that would be attributed to smaller-sized seeds.

Plant biomass of both of *P. hispida* and *E. polyandra*, increased in wide pots (Fig. 4-3), which suggested that both species prefer to grow in a thin soil. This is consistent with the reduction of growth under flood treatments (Chapter 3). *Pterostyrax hispida* and *E. polyandra* would extend horizontally their roots rather than vertically. This is consistent with some observations in the field studies: *E. polyandra* is distributed widely in landslide slopes, where their soils are very thin (Sakai & Ohsawa, 1993, 1994) and has well-developed fine lateral roots (Sakai, Sakai, & Akiyama, 1997). : Ito et al. (2008) reported some woody species with lateral roots are more often distributed in mounds of rock debris based on comparison of root architecture of current-year seedlings between species occurred in mountain regions. These studies suggest that that woody species to extend their roots horizontally would be able to establish distributed areas in riparianzones. The results that plant biomass increased in wide pots (Fig. 4-3) were consistent with previous observations.

This study experimentally showed that soil-particle size and pot diameter affected plant survival and growth under the same soil volume although previous study concluded that soil shape is less important than volume (McConnaughay, Berntson, & Bazzaz, 1993). More plants survived in wide pots, while most of plants died in narrow pots (Fig. 4-2). The biomass of both species was larger in wide pots than in narrow pots (Fig. 4-3). Therefore, I can conclude that *P. hispida* and *E. polyandra* can survive and grow even in thin soil if water supply is sufficient.

Table 4-1 Effects of pot diameter (D), soil-particle size (P) and their interaction (D:P) on difference in pot weight before and after watering.

Source	Chisq	Df	Pr
D	566.594	2	<0.001
P	230.861	1	<0.001
D*P	18.762	2	<0.001

Table 4-2 Effects of species (S), pot diameter (D), soil-particle size (P) and their interactions on the number of survival plants.

Source	Chisq	Df	Pr
Three-way analysis			
S	25.459	1	<0.001
D	52.593	2	<0.001
P	11.148	1	<0.001
S*D	9.510	2	0.009
S*P	2.967	1	0.085
D*P	1.220	2	0.543
S*D*P	2.116	2	0.347
Two-way analysis at *Pterostyrax hispida*			
D	37.360	2	<0.001
P	1.292	1	0.256
D*P	2.232	2	0.328
Two-way analysis at *Euptelea polyandry*			
D	25.877	2	<0.001
P	13.907	1	<0.001
D*P	1.105	2	0.576

Table 4-3 Effects of species (S), pot diameter (D), soil-particle size (P) and their interactions on the plant biomass.

Source	Chisq	Df	Pr
Three-way analysis			
S	0.945	1	0.331
D	122.467	2	<0.001
P	1.648	1	0.199
S*D	2.262	2	0.323
S*P	0.746	1	0.388
D*P	0.622	2	0.733
S*D*P	NA	0	NA
Two-way analysis at *Pterostyrax hispida*			
D	98.582	2	<0.001
P	0.881	1	0.348
D*P	0.835	2	0.659
Two-way analysis at *Euptelea polyandry*			
D	44.774	2	<0.001
P	1.814	1	0.178
D*P	NA	0	NA

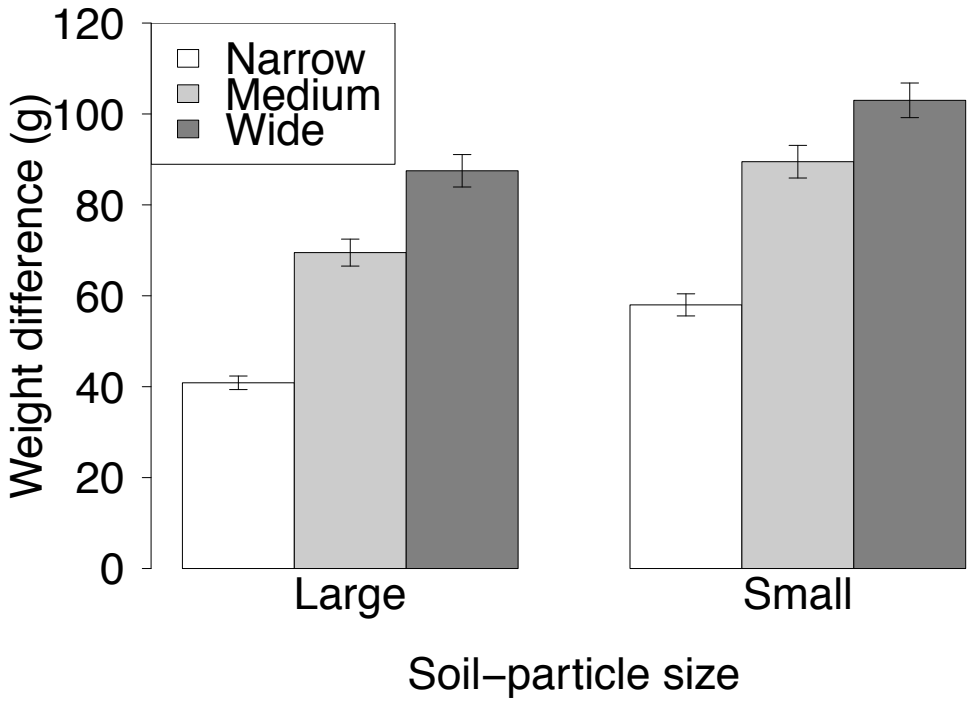

Fig. 4-1 Difference in pot weights between before and watering at different soil-particle size (large or small). White bar, light gray bar and dark gray bar shows narrow diameter, middle diameter and wide diameter, respectively.

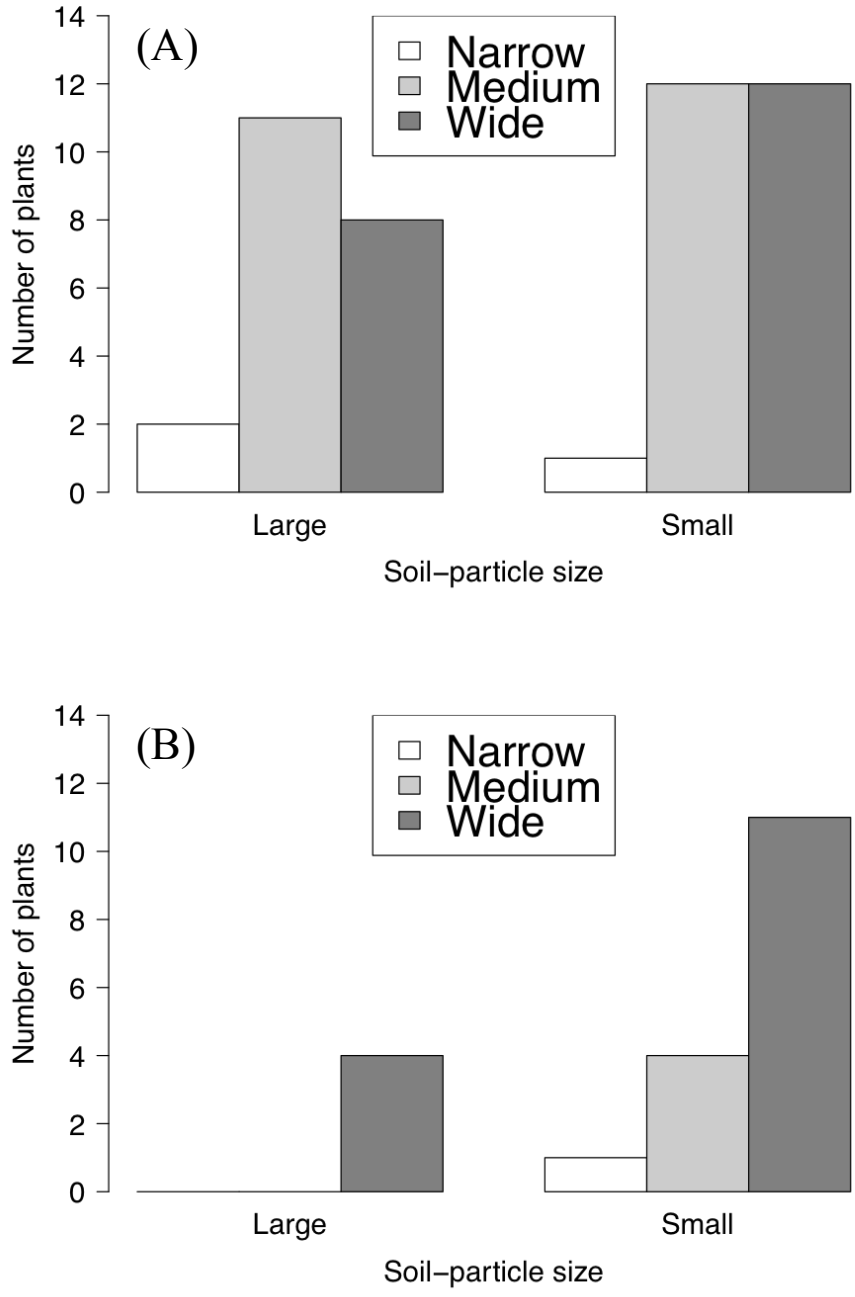

Fig. 4-2 Number of plants at the end of the experiment of (A) *Pterostyrax hispida* and (B) *Euptelea polyandra* at different soil-particle size (large or small). White bar, light gray bar and dark gray bar shows narrow diameter, middle diameter and wide diameter, respectively.

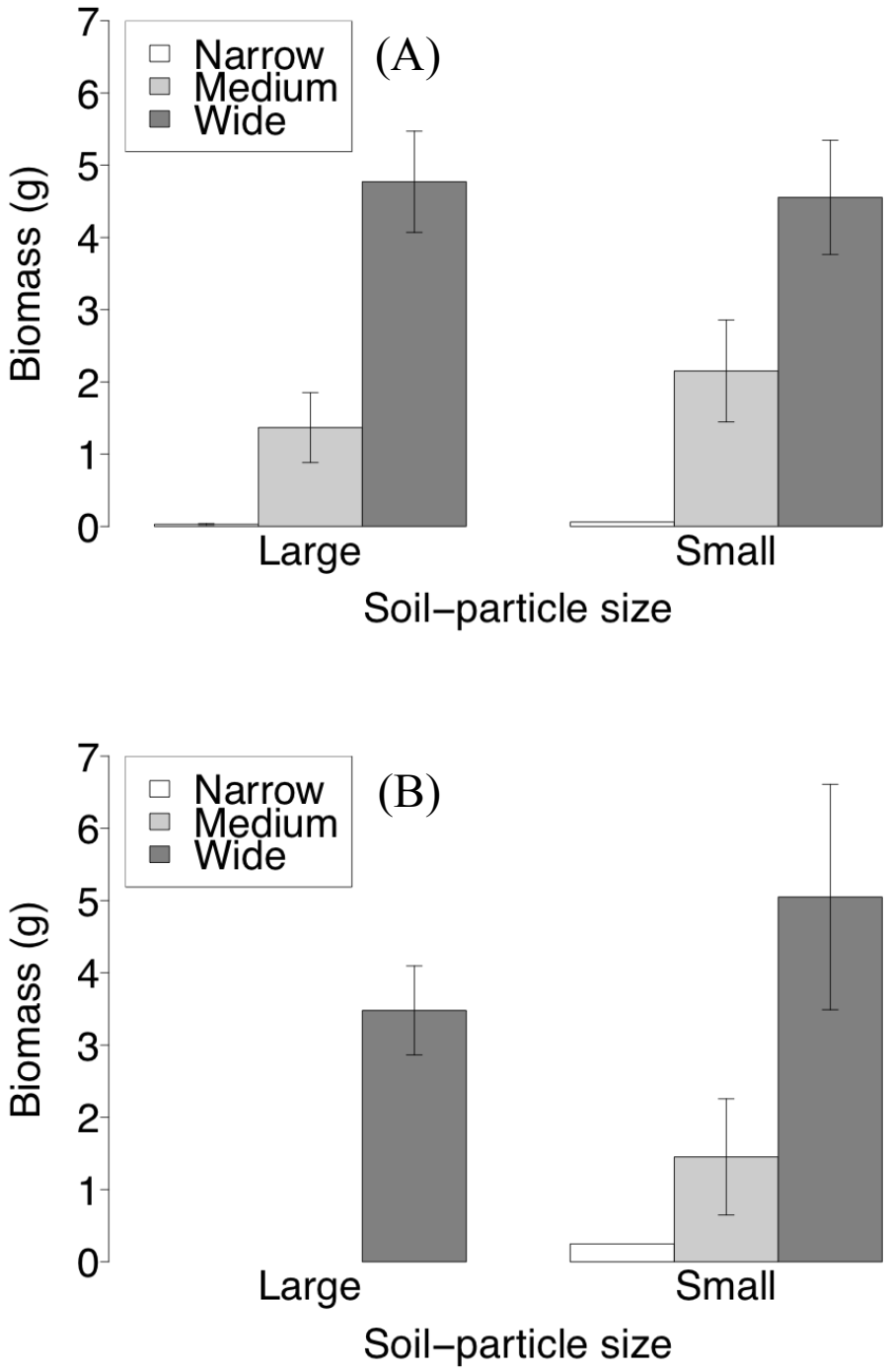

Fig. 4-3 Mean biomass (± SE) of (A) *Pterostyrax hispida* and (B) *Euptelea polyandra* at different soil-particle size (large or small). White bar, light gray bar and dark gray bar shows narrow diameter, middle diameter and wide diameter, respectively.

Fig. 4-4 The root architecture of *Pterostyrax hispida* in a pot with narrow diameter.

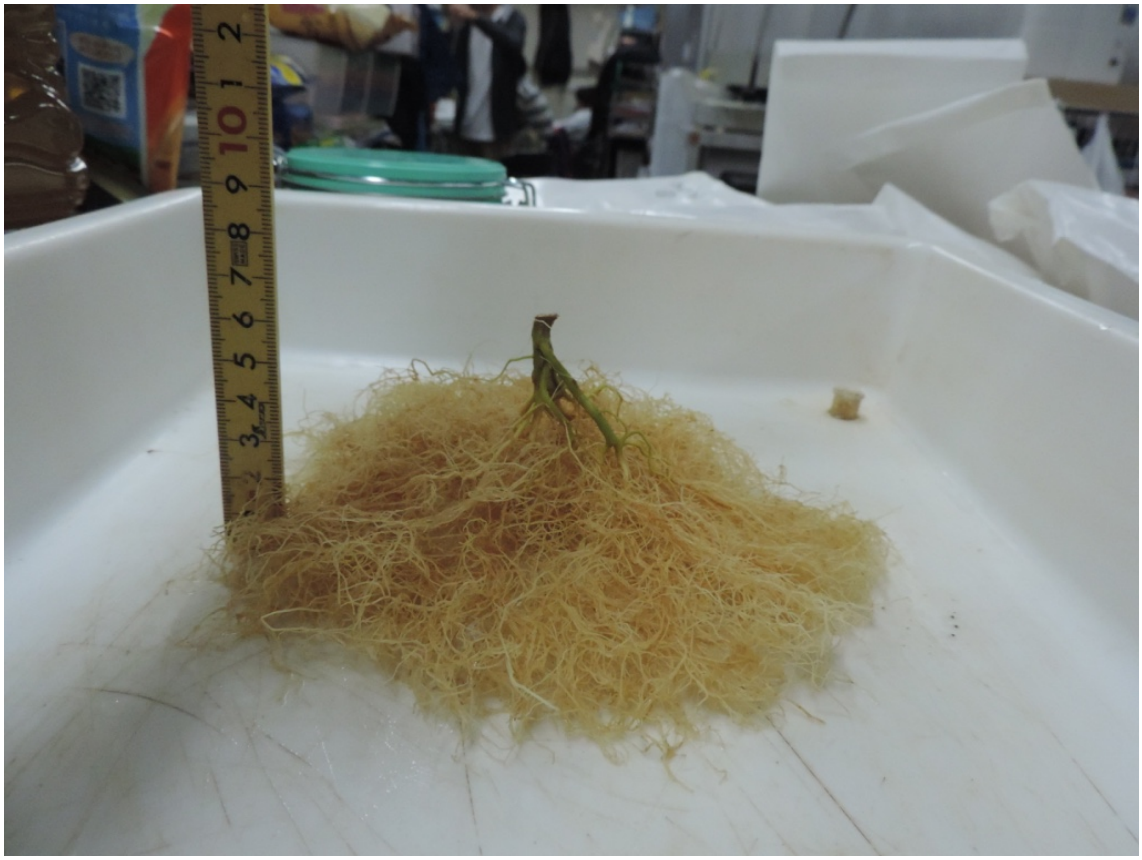

Fig. 4-5 The root architecture of *Pterostyrax hispida* in a pot with wide diameter.

Chapter 5

Conclusions

The field survey in Chapter 2 showed very low percentage of germination of *Pterostyrax hispida* although its seeds were able to germinate under various environments. The low germination would be compensated for regeneration by a large amount of dispersed seeds, which suggests the properties of *P. hispida* are consistent with r-selected species (Townsend et al., 2008). A rich production of seeds and their dispersal may be followed by increases in seedlings numbers for two or three years after the year of seed dispersal. Therefore, regeneration of *P. hispida* needs a large amount of seeds.

The field survey in Chapter 2 and garden experiments in Chapter 3 and Chapter 4 showed that seedlings of *P. hispida* preferred bright and aerobic conditions than shaded and submerge conditions. Numbers of seedlings or saplings in a quadrat increased along with increase in canopy openness. This trend is consistent with ecological properties of pioneer trees. Seedlings of *P. hispida* survived for submergence for a few days although floods reduced biomass increments. Seedlings established on thin soils under regular water supplied grew well though the soil lost moisture fast. This trend was consistent with the reduction of growth by the flooding. Therefore, the seedlings established on bright and well-drained sites would have higher chances to survive and grow.

More seedlings or saplings of *P. hispida* would be found at well-drain sites near mother trees. Distances from a mature tree would be important for seed dispersal because a mother tree of *P. hispida* produces wind dispersal seeds. Some quantity of dispersed seeds may stay in dormancy, and soil disturbances such as landslides would cause the germination of

dormant seeds in soil. Because seedlings of *P. hispida* had horizontally spreading roots, seedlings would establish on thin and well-drained soils.

Some more surveys are necessary to clarify the safe sites of *P. hispida*. First, we should know where seeds are actually dispersed. Second, seedling demography should be investigated in field conditions to test the tendencies obtained by my growth experiments. These surveys will contribute the understanding of population dynamics of *Pterostyrax hispida* in a mountain valley.

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